**DATA EVALUATION RECORD**
**CHRONIC SEDIMENT *Hyalella azteca* TOXICITY TEST**
**(FOLLOWING EPA TEST METHOD 100.4)**

1. **CHEMICAL:** Bifenthrin PC Code No.: 128825

2. **TEST MATERIAL:** Bifenthrin (tech.) Purity: 93.6%

3. **CITATION:**

| | |
|---|---|
| Authors: | Picard, C.R. |
| Title: | Bifenthrin – 42-Day Toxicity Test Exposing Freshwater Amphipods (*Hyalella azteca*) to Diquat Dibromide Applied to Sediment Under Static-Renewal Conditions Following EPA Test Methods. |
| Study Completion Date: | December 13, 2013 |
| Laboratory: | Smithers Viscient<br>790 Main Street<br>Wareham, MA 02571-1037 |
| Sponsor: | Consumer Specialty Products Association, Inc. for the<br>Bifenthrin Task Force Steering Committee/Joint Venture<br>1667 K Street, NW, Suite 300<br>Washington, DC 20006 |
| Laboratory Report ID: | 14011.6126 |
| MRID No.: | 49277501 |
| DP Barcode: | D418942 |

4. **REVIEWED BY:** Christie E. Padova, Staff Scientist, CSS-Dynamac Corporation

**Signature:** Christie E. Padova **Date:** 07/31/14

**APPROVED BY:** John Marton, Ph.D., Environmental Scientist, CDM Smith

**Signature:** **Date:** 06/01/15

5. **APPROVED BY:**

**Signature:** **Date:**

6. **STUDY PARAMETERS**

| | |
|---|---|
| **Scientific Name of Test Organism:** | *Hyalella azteca* |
| **Age of Test Organism:** | 8 days old |
| **Definitive Test Duration:** | 42 days |
| **Study Method:** | Intermittent flow-through |
| **Type of Concentrations:** | TWA bulk and OC-normalized sediment and TWA porewater concentrations |

## 7. CONCLUSIONS:

**Results Synopsis**

TWA SEDIMENT

NOAEC: 11 µg ai/kg
LOAEC: 24 µg ai/kg

TWA SEDIMENT, OC-NORMALIZED

NOAEC: 0.24 µg ai/g OC
LOAEC: 0.50 µg ai/g OC

TWA POREWATER

NOAEC: 0.044 µg ai/L
LOAEC: 0.076 µg ai/L

Endpoint(s) Affected: survival (Days 28, 35, and 42), cumulative offspring and offspring per female (Days 35 and 42)
Most Sensitive Endpoint(s): cumulative offspring and offspring per female (Days 35 and 42)

## 8. ADEQUACY OF THE STUDY:

A. Classification: Acceptable/Supplemental/Unacceptable

B. Rationale: This study followed methods described in the "Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates", 2nd Edition, Test Method 100.4: "*Hyalella azteca* 42-d Test for Measuring the Effects of Sediment-associated Contaminants on Survival, Growth, and Reproduction." March 2000 (EPA 600/R-99/064).

C. Reparability: N/A

## 9. MAJOR GUIDELINE DEVIATIONS:

This study does not fulfill any current U.S. EPA guideline requirement. Deviations from U.S. EPA Test Method 100.4 included:

- The temperature range of the overlying water exceeded recommendations (23 ± 1°C) on Days 1, 12, 13 and 14, with a maximum measurement of 26°C.
- Concentrations of $NH_3$-N in the overlying water varied by more than 50% (CVs ranged from 101 to 137%).

## 10. MATERIALS AND METHODS

### A. Test Organisms/Acclimation

| Guideline Criteria | Reported Information |
|---|---|
| **Species**<br>*Hyalella azteca* | *Hyalella azteca* |
| **Age of Test Organisms**<br>7- to 8-days old | 8 days old |
| **Source** | Laboratory cultures |
| **All organisms from the same source?** | Yes |
| **Culture Conditions** | Amphipod cultures were maintained under flow-through conditions in 20-L glass aquaria containing *ca.* 15 L of culture water (same source as overlying water used in definitive study). Nine days prior to test initiation, a sub-culture of reproducing adults was transferred to 9.5-L aquaria (isolation tanks) containing 8 L of culture water. Juvenile amphipods (<24 hours old) produced from the isolated adults were pipetted into 1-L beakers containing *ca.* 0.80 L of laboratory well water. During the holding period, DO ranged from 7.0 to 8.0 mg/L and temperature ranged from 24 to 26 °C. |

| Guideline Criteria | Reported Information |
|---|---|
| **Feeding** | Amphipods were fed once daily a combination of yeast, cereal leaves and flaked fish food suspension (YCT, 100 mg/mL). A small amount of *Ankistrodesmus falcatus*, a unicellular green algae, was also added at the start of acclimation to provided a supplemental food source. |
| **Pre-test Mortality** | Test organisms appeared healthy, with no mortality observed during the 48-hour period prior to test initiation. |

**B. Test System**

| Guideline Criteria | Reported Information |
|---|---|
| **Dilution water (overlying water)** | Laboratory well water characterized as having a total hardness and total alkalinity of 38 to 48 and 20 to 22 mg/L as $CaCO_3$, respectively, a pH range of 7.3 to 7.8, and a conductivity range of 230 to 350 µS/cm.<br><br>In addition, the TOC of the well water was 0.31 to 1.3 mg/L for July through September 2013. |
| **Does water support test animals without observable signs of stress?** | Yes |
| **Water Temperature**<br>Mean daily: 23 ± 1°C<br>Instantaneous: 23 ± 3°C | Daily: 22 to 26°C<br>Continuous: 21 to 26°C |
| **pH** | 6.7 to 7.4 |
| **Dissolved Oxygen**<br>>2.5 mg/L | 4.9 to 8.6 mg/L |

| Guideline Criteria | Reported Information |
|---|---|
| **Ammonia**<br>Should not vary more than 50%. | ≤0.10 to 0.85 mg/L as N<br><br>CVs of ammonia concentrations exceeded 100% for all treatment levels. |
| **Hardness**<br>Should not vary more than 50%. | 36 to 64 mg/L as $CaCO_3$ |
| **Alkalinity**<br>Should not vary more than 50%. | 16 to 20 mg/L as $CaCO_3$ |
| **Conductivity** | 220 to 330 µhmos/cm |
| **Test Sediment** | Natural freshwater sediment (Batch No. 040213) was collected from Glen Charlie Pond, Wareham, MA and wet-press sieved (2.0 mm) to remove large particles and indigenous organisms. |
| **Sediment Characterization** | Particle distribution – 83% sand, 12% silt, 5% clay<br>TOC – 4.8%<br>Percent solids – 23.91%<br>pH – 5.4<br>Pore water ammonia concentration – 4.9 mg/L (as N) |

| Guideline Criteria | Reported Information |
|---|---|
| **Test Materials** | Bifenthrin technical<br>IUPAC name: 2-methylbiphenyl-3-ylmethyl (1*RS*,3*RS*)-3-[(*Z*)-2-chloro-3,3,3-trifluoroprop-1-enyl]-2,2-dimethylcyclopropanecarboxylate<br>CAS name: (2-methyl[1,1′-biphenyl]-3-yl)methyl (1*R*,3*R*)-*rel*-3-[(1*Z*)-2-chloro-3,3,3-trifluoro-1-propen-1-yl]-2,2-dimethylcyclopropanecarboxylate<br>CAS No.: 82657-04-3<br>Description: liquid<br>Lot no.: PL09-0251<br>Purity: 93.6%<br>Storage: room temperature, dark<br><br>A 25 µg/mL primary stock solution was prepared by bringing 0.00264 g of test substance (0.00247 g bifenthrin) to 100 mL with acetone. The primary stock was clear and colorless with no visible un-dissolved test substance. Five individual dosing stock solutions were prepared (at 0.559 to 8.87 µg/mL) by diluting the appropriate volume of primary stock into 25 mL acetone. Dosing stock solutions were clear and colorless with no visible un-dissolved test substance. |
| **Solvents** | Acetone, 10 mL/0.8869 kg dw<br><br>The acetone was allowed to completely evaporate during the mixing procedure.<br><br>Both solvent control and negative control groups were included in the study. |

| Guideline Criteria | Reported Information |
|---|---|
| **Sediment Spiking** | A jar-rolling technique was used to apply the test substance to the sediment. A 10-mL volume of the appropriate prepared dosing stock solution (in acetone) was applied to 0.050 kg of fine silica sand and the solvent was allowed to evaporate off for 60 minutes. The dry sand was then added to 3.5 kg of wet sediment (total of 0.8869 kg dw) in individual glass jars. Each jar was then rolled at room temperature for 4 hours at *ca*. 15 rpm.<br><br>The range of nominal concentrations (6.3 to 100 µg/kg) was based upon the results of a preliminary range-finding study and in consultation with the Sponsor. |
| **Sediment Conditioning** | The jars containing treated sediment were stored upright at 2 to 8°C for a 14-day equilibration period.<br><br>Once a week during the equilibration period and prior to being added into the replicate exposure vessels, the jars were mixed on the rolling mill at room temperature for 2 hours to ensure the sediment was homogeneous. |

| Guideline Criteria | Reported Information |
|---|---|
| **Test Vessels**<br>300 mL high-form lipless glass beakers containing 100 mL of sediment and 175 ml of overlying water | 300-mL glass vessels, each with two slots cut on the top edge covered with 40-mesh Nitex® screen for drainage.<br><br>Through Day 28: each vessel contained 100 mL (*ca.* 4.0-cm layer) of sediment (equivalent to 26.5 g dw) and 175 mL of overlying water. The total overlying water plus sediment volume was maintained at approx. 275 mL.<br><br>Days 28 to 42: each vessel contained 300 mL water only and a 3 cm x 3 cm piece of nylon Nitex® screen. |
| **Type of Dilution System**<br>Continuous or intermittent | Intermittent flow-through |
| **Flow Rate**<br>2 volume additions/day | 2 volume additions/day |
| **Aeration**<br>None, unless DO in overlying water drops <2.5 mg/L | None reported |
| **Photoperiod**<br>16 hours light, 8 hours dark using wide-spectrum fluorescent lights; intensity of 100 to 1000 lux | 16-hour light/8-hour dark photoperiod using fluorescent bulbs at an intensity range of 220 to 710 lux. |
| **Feeding** | Amphipods were fed a combination of yeast, cereal leaves and flaked fish food suspension (YCT, 100 mg/mL) at a rate of 1.0 mL YCT per replicate per day. |

**C. Test Design**

| Guideline Criteria | Reported Information |
|---|---|
| **Duration**<br>42 days | 42 days |
| **Sediment Into Test Chambers**<br>One day prior (Day -1) to start of test: each sediment should be thoroughly homogenized and added to test chambers; Overlying water is added to chambers in a manner that minimizes suspension of sediment. | Test systems were established on Day -1. Overlying water was gently added using a turbulence reducer, and each vessel was placed under the renewal system. |
| **Renewal of Overlying Water**<br>Renewal of the overlying water should be conducted on day -1 prior to the addition of organisms or food on day 0. For flow-through systems, the flow rates should not vary by more than 10% between any two chambers at any time. Proper operation should be verified by calibration prior to test initiation. | The overlying water was renewed via an intermittent delivery system in combination with a calibrated water-distribution system. The test system was calibrated before and after the test, and visually inspected at least twice daily for proper functioning. |
| **Monitoring the test**<br>All test chambers should be checked daily and observations made to assess organism behavior such as sediment avoidance. | Test systems were observed daily for organism behavior (e.g., adverse effects) and characteristics of test solutions. |

| Guideline Criteria | Reported Information |
|---|---|
| **Range Finding Test**<br>A definitive test will not be required if no toxicity is observed at concentrations of 100 mg/kg dry weight of sediment**.** | Preliminary toxicity assessment<br>• 42-day exposure at nominal levels of 0 (negative and solvent controls), 0.27, 1.1, 4.4, 18 and 70 µg/kg<br>• Ten, *ca.* 8-day old amphipods per replicate, with eight replicates per level<br>• Day-28 survival averaged 88, 98, 99, 100, 93, 86 and 1%, respectively; the difference was significant ($p<0.05$) at the 70 µg/kg level (excluded from further statistical analysis).<br>• No further effects observed for the following endpoints (up to 18 µg/kg): Day-28 length (5.27 to 5.51 mm), Day-35 survival (66 to 100%), Day-35 offspring released per female (2.6 to 6.6), Day-42 survival (78 to 98%), Day-42 length (5.17 to 5.99 mm), Day-42 offspring released per female (3.5 to 9.5), and Day-42 male:female ratio (0.74 to 1.7). |
| **Nominal Sediment Concentrations** | 0 (negative and solvent controls), 6.3, 13, 25, 50 and 100 µg/kg dw sediment |
| **Number of Test Organisms**<br>10 organisms per chamber are recommended; 12 replicates per treatment should be used (4 for 28-day survival and 8 for 35- and 42-day survival, growth, and reproduction) | 10 organisms per test chamber<br><br>12 biological replicates per level |
| **Test organisms randomly or impartially assigned to test vessels?** | Yes |

| Guideline Criteria | Reported Information |
|---|---|
| **Water Parameter Measurements**<br>Conductivity, hardness, alkalinity, and ammonia should be measured in all treatments at the beginning and end of the sediment exposure (Days 0 and 28). Conductivity should also be determined weekly.<br><br>DO and pH should be measured at least three times per week.<br><br>Temperature should be measured daily in at least one chamber from each level, and monitored continuously in the water bath. | For all levels (as applicable), total hardness, alkalinity, conductivity and ammonia concentrations were measured in a composite sample of the overlying water on Days 0, 28, 29, and 42.<br><br>DO, temperature, and pH were measured in the overlying water of each replicate vessel on Days 0, 28, 29, and 42. On the remaining days, DO and temperature were measured in one alternating replicate from each level, and the temperature was continuously monitored in an auxiliary vessel in the water bath. |
| **Chemical Analysis** | Concentrations of bifenthrin were determined in sediment and pore water from surrogate test vessels collected on Days 0, 14, and 28 (termination of sediment phase of exposure).<br><br>The overlying water was decanted and discarded, and the sediment was centrifuged at *ca.* 10,000 *g* for 30 minutes to isolate the sediment and pore water matrices.<br><br>Sediment and pore water samples were analyzed using liquid chromatography with mass spectrometry (LC/MS/MS) and gas chromatography with mass selective detection (GC/MSD), respectively, based on methodology validated at Smithers Viscient. |

## 11. REPORTED RESULTS

### A. General Results

| Guideline Criteria | Reported Information |
|---|---|
| **Quality assurance and GLP compliance statements were included in the report?** | Yes. This study was conducted in accordance with GLP Standards as specified in 40 CFR 160 with the following exceptions: routine water, sediment and food contaminant screening analyses for pesticides, PCBs, and toxic metals. These analyses, however, were performed using certified laboratories and standard validated methods. |
| **Control Criteria**<br>**Was average survival on Day 28 ≥80%**<br><br>**Was average growth on Day 28 ≥0.15 mg (dry weight) and/or ≥3.2 mm (length) per amphipod?**<br><br>**Did controls produce ≥2 offspring per female between Days 28 and 42?** | All criteria met:<br>Negative control: 95%<br>Solvent control: 93%<br><br>Negative control: 4.86 mm per amphipod<br>Solvent control: 4.87 mm per amphipod<br><br>Negative control: 9.5 offspring per female<br>Solvent control: 9.1 offspring per female |

| Guideline Criteria | Reported Information |
|---|---|
| **Percent Recovery of Chemical** | Procedural recoveries (from QC samples) conducted concurrently with sample analysis:<br><br>Sediment:<br>Recovery range of 84.9 to 110% (n=9)<br>LOQ $_{sediment}$ = 0.35 to 0.42 µg ai/kg<br><br>The reviewer-calculated time-weighted average sediment concentrations were 4.9, 11, 24, 49, and 95 µg ai/kg, which corresponded to 0.10, 0.24, 0.50, 1.0, and 2.0 µg ai/g OC.<br><br>Aqueous:<br>Recovery range of 70.8 to 117% (n=8) (excludes one outlier of 12.0%)<br>LOQ $_{water}$ = 0.0074 to 0.0083 µg ai/L<br><br>The reviewer-calculated time-weighted porewater concentrations were 0.045, 0.044, 0.076, 0.10, and 0.18 µg ai/L. |
| **Data Endpoints** | Day 28:<br>- Survival<br>- Length<br>Day 35:<br>- Survival<br>- Offspring per female<br>Day 42:<br>- Survival<br>- Length<br>- Offspring per female<br>- Male:female ratio |
| **Raw data included?** | Yes, sufficient. |

Effects Data:

28-Day Endpoints:

After 28 Days, survival averaged 95 and 93% for the negative and solvent control levels, respectively, and 78, 92, 89, 12 and 0% for the mean-measured 5.0, 11, 22, 47 and 91 µg ai/kg treatment levels, respectively. Differences were statistically-significant compared to the negative control at the 5.0, 47 and 91 µg ai/kg levels ($p<0.05$; Steel's Many-One Rank Sum Test). However, the effect observed at the 5.0 µg ai/kg level was not concentration-responsive and therefore not considered to be a result of treatment (the lower mean survival was driven by two replicates that had 30% and 40% survival). Due to the effect on survival at the 47 and 91 µg ai/kg concentrations, these levels were excluded from further statistical analysis. The subsequent NOAEC and LOAEC for 28-Day survival were 22 and 47 µg ai/kg, respectively. The 28-Day $LC_{50}$ (with 95% C.I.) was 33 (31 to 34) µg ai/kg. Adjusted for the organic carbon (OC) content of the sediment (i.e., 4.8%), the NOAEC and LOAEC for 28-Day survival were 0.46 and 0.98 µg ai/g OC, respectively, and the $LC_{50}$ (with 95% C.I.) was 0.68 (0.65 to 0.71) µg ai/g OC.

Day-28 lengths averaged 4.86 and 4.87 mm per amphipod at the negative and solvent control levels, respectively, and 5.01, 4.77, 4.33 and 5.44 mm per amphipod at the mean-measured 5.0, 11, 22 and 47 µg ai/kg treatment levels, respectively. The difference was statistically-significant compared to the negative control at the 22 µg ai/kg level ($p<0.05$; Dunnett's Multiple Comparison Test). No concentration resulted in ≥50% reduction in length compared to the negative control, and therefore the NOAEC, LOAEC and observed $EC_{50}$ for 28-Day length were 11, 22 and >47 µg ai/kg, respectively. Adjusted for the organic carbon (OC) content of the sediment (i.e., 4.8%), the NOAEC, LOAEC, and $EC_{50}$ for 28-Day growth were 0.23, 0.46 and >0.98 µg ai/g OC, respectively.

| Toxicant Conc., Sediment (µg ai/kg dw) | | Day 28 | |
|---|---|---|---|
| Nominal | Mean-measured | Survival (% ± SD) | Length (mg/amphipod ± SD) |
| Ctrl | <LOQ[a] | 95 ± 5 | 4.86 ± 0.16 |
| Solvent Ctrl | <LOQ[a] | 93 ± 9 | 4.87 ± 0.093 |
| 6.3 | 5.0 | 78 ± 23* | 5.01 ± 0.36 |
| 13 | 11 | 92 ± 8 | 4.77 ± 0.34 |
| 25 | 22 | 89 ± 11 | 4.33 ± 0.20* |
| 50 | 47 | 12 ± 10* | 5.44 ± 0.65[b] |
| 100 | 91 | 0 ± 0* | N/A[b] |

* Statistically-significant reduction compared to the negative control ($p<0.05$).
[a] LOQ = 0.35 to 0.42 µg ai/kg.

(b) Data not subjected to statistical analysis due to a significant effect at this level on 28-Day survival.

35-Day Endpoints:

Survival at Day 35 averaged 90 and 88% for the negative and solvent control levels, respectively, and 69, 89, 83, and 15% for the mean-measured 5.0, 11, 22, and 47 µg ai/kg treatment levels, respectively. Although the difference was statistically-significant compared to the negative control at the 5.0 µg ai/kg level ($p<0.05$; Dunnett's Multiple Comparison Test), it was not concentration-responsive and not considered to be treatment-related (the lower mean survival was driven by two replicates that had 30% survival). The subsequent NOAEC and LOAEC for 35-Day survival were 22 and >22 µg ai/kg, respectively. The 35-Day $LC_{50}$ (with 95% C.I.) was 33 (31 to 35) µg ai/kg. Adjusted for the organic carbon (OC) content of the sediment (i.e., 4.8%), the NOAEC and LOAEC for 35-Day survival were 0.46 and >0.46 µg ai/g OC, respectively, and the $LC_{50}$ (with 95% C.I.) was 0.69 (0.65 to 0.74) µg ai/g OC.

Reproduction, assessed as the number of offspring released per female amphipod, averaged 5.4 and 4.5 for the negative and solvent control levels, respectively, and 2.6, 3.3, and 0.93 for the mean-measured 5.0, 11 and 22 µg ai/kg treatment levels, respectively. Statistical analysis determined significance ($p<0.05$) at the 5.0 and 22 µg ai/kg levels. However, the effect observed at the 5.0 µg ai/kg level was not concentration-responsive, and the lower mean offspring production was driven by low reproduction in the replicates with lower survival. The 35-Day $EC_{50}$ (with 95% C.I.) for reproduction was 13 (2.0 to 19) µg ai/kg, and the NOAEC and LOAEC were 11 and 22 µg ai/kg, respectively. Adjusted for the organic carbon (OC) content of the sediment (i.e., 4.8%), the NOAEC and LOAEC for 35-Day reproduction were 0.23 and 0.46 µg ai/g OC, respectively, and the $EC_{50}$ (with 95% C.I.) was 0.30 (0.069 to 0.43) µg ai/g OC.

| Toxicant Conc., Sediment (µg ai/kg dw) | | Day 35 | |
|---|---|---|---|
| Nominal | Mean-measured | Survival (% ± SD) | Reproduction (Offspring Released per Female ± SD) |
| Ctrl | <LOQ(a) | 90 ± 11 | 5.4 ± 2.0 |

| | | | |
|---|---|---|---|
| Solvent Ctrl | <LOQ[a] | 88 ± 12 | 4.5 ± 2.7 |
| 6.3 | 5.0 | 69 ± 25* | 2.6 ± 2.7* |
| 13 | 11 | 89 ± 11 | 3.3 ± 1.0 |
| 25 | 22 | 83 ± 18 | 0.93 ± 1.5* |
| 50 | 47 | 15 ± 8[b] | N/A[b] |
| 100 | 91 | N/A[b] | N/A[b] |

* Statistically-significant reduction compared to the negative control ($p<0.05$).
[a] LOQ = 0.35 to 0.42 µg ai/kg.
[b] Data not subjected to statistical analysis due to a significant effect at this level on 28-Day survival.

42-Day Endpoints:

Survival at Day 42 averaged 86 and 84% for the negative and solvent control levels, respectively, and 66, 88, 79, and 15% for the mean-measured 5.0, 11, 22, and 47 µg ai/kg treatment levels, respectively. Although the difference was statistically-significant compared to the negative control at the 5.0 µg ai/kg level ($p<0.05$; Dunnett's Multiple Comparison Test), it was not concentration-responsive and not considered to be treatment-related (the lower mean survival was driven by two replicates that had 30% survival). The subsequent NOAEC and LOAEC for 42-Day survival were 22 and >22 µg ai/kg, respectively. The 35-Day $LC_{50}$ (with 95% C.I.) was 33 (31 to 36) µg ai/kg. Adjusted for the organic carbon (OC) content of the sediment (i.e., 4.8%), the NOAEC and LOAEC for 42-Day survival were 0.46 and >0.46 µg ai/g OC, respectively, and the $LC_{50}$ (with 95% C.I.) was 0.70 (0.65 to 0.74) µg ai/g OC.

Lengths of amphipods on Day 42 averaged 5.61, 5.61, 5.75, 5.75, 5.52 and 6.30 mm per amphipod for the mean-measured 0 (negative control), 0 (solvent control), 5.0, 11, 22, and 47 µg ai/kg treatment levels, respectively, with no statistically-significant differences indicated. Since no concentration resulted in ≥50% reduction in length, the subsequent NOAEC, LOAEC and observed $EC_{50}$ for 42-Day length were 22, >22 and >47 µg ai/kg, respectively. Adjusted for the organic carbon (OC) content of the sediment (i.e., 4.8%), the NOAEC, LOAEC, and $EC_{50}$ for 42-Day growth were 0.46, >0.46 and >0.98 µg ai/g OC, respectively.

Reproduction, assessed as the number of offspring released per female amphipod, averaged 9.5 and 9.1 for the negative and solvent control levels, respectively, and 8.7, 8.0 and 3.1 for the mean-measured 5.0, 11 and 22 µg ai/kg treatment levels, respectively. Statistical analysis determined significance ($p<0.05$) at the 22 µg ai/kg treatment level, and the subsequent NOAEC and LOAEC were 11 and 22 µg ai/kg, respectively. The 42-Day $EC_{50}$ for reproduction was 18 µg ai/kg, with a lower 95% confidence limit of 14 µg ai/kg (an upper 95% confidence limit could not be determined). Adjusted for the organic carbon

(OC) content of the sediment (i.e., 4.8%), the NOAEC and LOAEC for 42-Day reproduction were 0.23 and 0.46 µg ai/g OC, respectively, and the $EC_{50}$ (with 95% C.I.) was 0.38 (0.31 to not calculable) µg ai/g OC.

| Toxicant Conc., Sediment (µg ai/kg dw) | | Day 42 | | | |
|---|---|---|---|---|---|
| Nominal | Mean-measured | Survival (% ± SD) | Length (mm/ amphipod) | Reproduction (Offspring Released per Female ± SD) | Male:Female Ratio (± SD) |
| Ctrl | <LOQ[a] | 86 ± 13 | 5.61 ± 0.33 | 9.5 ± 2.0 | 1.7 ± 1.0 |
| Solvent Ctrl | <LOQ[a] | 84 ± 14 | 5.61 ± 0.23 | 9.1 ± 4.0 | 2.0 ± 2.7 |
| 6.3 | 5.0 | 66 ± 23* | 5.75 ± 0.22 | 8.7 ± 6.1 | 1.0 ± 0.51 |
| 13 | 11 | 88 ± 14 | 5.75 ± 0.35 | 8.0 ± 2.4 | 1.2 ± 0.77 |
| 25 | 22 | 79 ± 17 | 5.52 ± 0.40 | 3.1 ± 3.0* | 1.5 ± 1.1 |
| 50 | 47 | 15 ± 8[b] | 6.30 ± 1.3[b] | N/A[b] | 0 ± 0[b] |
| 100 | 91 | N/A[b] | N/A[b] | N/A[b] | N/A[b] |

* Statistically-significant reduction compared to the negative control ($p<0.05$).
[a] LOQ = 0.35 to 0.42 µg ai/kg.
[b] Data not subjected to statistical analysis due to a significant effect at this level on 28-Day survival.

No statistically-significant differences from the negative control were indicated at any level up to and including 22 µg ai/kg regarding male:female ratio (Day 42), which averaged 1.7, 2.0, 1.0, 1.2, and 1.5 for the negative control, solvent control, and mean-measured 5.0, 11, and 22 µg ai/kg treatment levels, respectively. The NOAEC and LOAEC for male:female ratio were 22 and >22 µg ai/kg, respectively. Adjusted for the organic carbon (OC) content of the sediment (i.e., 4.8%), the NOAEC and LOAEC for male:female ratio were 0.46 and >0.46 µg ai/g OC, respectively.

Analytical:

Dosing stock solutions and treated sediment from all levels (prior to allocation into the replicate vessels) were analyzed for bifenthrin. Recoveries in the stock solutions ranged from 110 to 130% of nominal concentrations. Analysis of the spiked sediment following dosing and prior to allocation into the replicate exposure vessels ranged from 69 to 130% of nominal concentrations.

During testing, concentrations of bifenthrin were determined in sediment and pore water on Days 0, 14 and 28. Results demonstrated that the majority of the test substance applied to the sediment remained associated with the sediment, with low concentrations of the test substance detected in the aqueous matrix.

For the nominal 6.3, 13, 25, 50 and 100 µg/kg levels, sediment concentrations measured 6.6, 12, 18, 40 and 78 µg ai/kg, respectively, on Day 0; 4.2, 12, 30, 55 and 110 µg ai/kg, respectively, on Day 14; and 4.4, 9.7, 18, 46 and 82 µg ai/kg, respectively, on Day 28. Calculated mean-measured concentrations represented 80 to 94% of nominal sediment levels.

In pore water, maximum concentrations were observed on Day 0. For the nominal 6.3, 13, 25, 50 and 100 µg/kg levels, pore water concentrations measured 0.13, 0.088, 0.12, 0.10 and 0.19 µg ai//L, respectively, on Day 0; 0.018, 0.032, 0.062, 0.12, and 0.17 µg ai/L, respectively, on Day 14; and 0.014, 0.024, 0.059, 0.068 and 0.20 µg ai/L, respectively, on Day 28.

**B. Statistical Results (From Study Report)**

Endpoints that were statistically-analyzed included survival and growth (length) on Day 28; survival and reproduction (offspring per female) on Day 35; and survival, growth, reproduction, and male:female ratio on Day 42. Analyses were performed with CETIS™ (version 1.8. 2013) statistical software. Percent survival data were arcsine square-root transformed prior to analysis. Results were provided in terms of mean-measured sediment concentrations and OC-normalized sediment concentrations (see Reviewer's Comments section).

An Equal Variance Two-Sample t-Test or Wilcoxon's Rank Sum Two-Sample Test was used to compare the performance of the negative control and solvent control data. Regardless of the results of the comparisons (not reported), the treatment groups were compared to the negative control data to determine potential treatment-related effects.

For all endpoints, the data were tested for normality using the Shapiro-Wilks' Test, and for homogeneity of variance using Bartlett's Test. All endpoints met the assumption for Day-28 survival data did not meet the assumption for homogeneity of variance and were analyzed using Steel's Many-One Rank Test. Remaining endpoints met both assumptions, and were evaluated using Dunnett's Multiple Comparison Test or Bonferroni's Adjusted t-Test. NOAEC and LOAEC values were assigned based on significance. All statistical analyses were conducted at the 95% level of certainty except in the case of the qualification tests (i.e., Shapiro-Wilks' and Bartlett's Tests), in which a 99% level of certainty was applied.

The Trimmed Spearman-Karber method within CETIS™ was used to calculate the $LC_{50}$ values and associated 95% confidence intervals (C.I.) for Days 28, 35 and 42. The Linear Interpolation method (within CETIS™) was used to calculate any relevant $EC_{50}$ values.

| Endpoint | Methods | Mean-measured Sediment, µg ai/kg | OC-normalized Sediment, µg ai/g OC |
|---|---|---|---|
| 28-Day survival | Steel's Many-One Rank Test | NOAEC: 22<br>LOAEC: 47<br>$LC_{50}$: 33<br>95% C.I.: 31 to 34 | NOAEC: 0.46<br>LOAEC: 0.98<br>$LC_{50}$: 0.68<br>95% C.I.: 0.65 to 0.71 |
| 28-Day length | Dunnett's Multiple Comparison Test | NOAEC: 11<br>LOAEC: 22<br>$EC_{50}$: >47<br>95% C.I.: N/A | NOAEC: 0.23<br>LOAEC: 0.46<br>$EC_{50}$: >0.98<br>95% C.I.: N/A |
| 35-Day survival | Dunnett's Multiple Comparison Test | NOAEC: 22<br>LOAEC: >22<br>$LC_{50}$: 33<br>95% C.I.: 31 to 35 | NOAEC: 0.46<br>LOAEC: >0.46<br>$LC_{50}$: 0.69<br>95% C.I.: 0.65 to 0.74 |
| 35-Day offspring per female | Bonferroni's Adjusted t-Test | NOAEC: 11<br>LOAEC: 22<br>$EC_{50}$: 13<br>95% C.I.: 2.0 to 19 | NOAEC: 0.23<br>LOAEC: 0.46<br>$EC_{50}$: 0.30<br>95% C.I.: 0.069 to 0.43 |
| 42-Day survival | Dunnett's Multiple Comparison Test | NOAEC: 22<br>LOAEC: >22<br>$LC_{50}$: 33<br>95% C.I.: 31 to 36 | NOAEC: 0.46<br>LOAEC: >0.46<br>$LC_{50}$: 0.70<br>95% C.I.: 0.65 to 0.74 |
| 42-Day length | Dunnett's Multiple Comparison Test | NOAEC: 22<br>LOAEC: >22<br>$EC_{50}$: >47<br>95% C.I.: N/A | NOAEC: 0.46<br>LOAEC: >0.46<br>$EC_{50}$: >0.98<br>95% C.I.: N/A |
| 42-Day offspring per female | Bonferroni's Adjusted t-Test | NOAEC: 11<br>LOAEC: 22<br>$EC_{50}$: 18<br>95% C.I.: 14 to N/D | NOAEC: 0.23<br>LOAEC: 0.46<br>$EC_{50}$: 0.38<br>95% C.I.: 0.31 to N/D |

| Endpoint | Methods | Mean-measured Sediment, µg ai/kg | OC-normalized Sediment, µg ai/g OC |
|---|---|---|---|
| 42-Day male:female ratio | Bonferroni's Adjusted t-Test | NOAEC: 22<br>LOAEC: >22 | NOAEC: 0.46<br>LOAEC: >0.46 |

N/D – A corresponding upper 95% C.I. limit could not be determined.

Endpoint(s) Affected: survival (Day 28), growth (Day 28), and reproduction (Days 35 and 42)
Most Sensitive Endpoint(s): growth (Day 28) and reproduction (Days 35 and 42)

## 12. VERIFICATION OF STATISTICAL RESULTS

Statistical Method: The reviewer analyzed survival (Days 28, 35, and 42), reproduction as cumulative offspring and cumulative offspring per female (Days 35 and 42), length (Days 28 and 42), and male:female (Day 42) using CETIS statistical software version 1.8.7.12 with database backend settings implemented by EFED on 3/25/14. Negative and solvent control data were compared for each endpoint using a two-sample t-test assuming equal variance. No differences were detected between the controls and all subsequent analyses were conducted by comparing treatment data to the negative control only. Data were then tested for normality using the Shapiro-Wilk test ($\alpha = 0.01$) and for homogeneity of variance using either the Bartlett ($\alpha = 0.01$) or Levene test ($\alpha = 0.01$). Offspring per female and length on Days 35 met these assumptions and were analyzed using ANOVA followed by the Dunnett Multiple Comparison test. Day 42 reproductive endpoint data did not satisfy parametric assumptions, but exhibited dose-dependent responses and were analyzed using the Jonckheere-Terpstra Step-Down test. All remaining endpoints were analyzed using the non-parametric Mann-Whitney U Two Sample test. Male:female was analyzed for both significant increases and decreases relative to the negative control. Analyses were conducted using the reviewer-calculated time-weighted average concentrations, but were converted to the TWA OC-normalized sediment and TWA porewater concentrations.

| Endpoint | Methods | TWA Sediment (µg ai/kg) | TWA OC Sediment (µg ai/g OC) | TWA Porewater (µg ai/L) |
|---|---|---|---|---|
| Day 28 Survival | Mann-Whitney | NOAEL: 24<br>LOAEL: 49 | NOAEL: 0.50<br>LOAEL: 1.0 | NOAEL: 0.076<br>LOAEL: 0.10 |
| Day 28 Length | Dunnett | NOAEL: 49<br>LOAEL: >49 | NOAEL: 1.0<br>LOAEL: >1.0 | NOAEL: 0.10<br>LOAEL: >0.10 |
| Day 35 Survival | Mann-Whitney | NOAEL: 24<br>LOAEL: 49 | NOAEL: 0.50<br>LOAEL: 1.0 | NOAEL: 0.076<br>LOAEL: 0.10 |
| Day 35 Offspring/Fem | Dunnett | NOAEL: 11<br>LOAEL: 24 | NOAEL: 0.24<br>LOAEL: 0.50 | NOAEL: 0.044<br>LOAEL: 0.076 |
| Day 35 Cum. Off. | Mann-Whitney | NOAEL: 11<br>LOAEL: 24 | NOAEL: 0.24<br>LOAEL: 0.50 | NOAEL: 0.044<br>LOAEL: 0.076 |
| Day 42 Survival | Mann-Whitney | NOAEL: 24<br>LOAEL: 49 | NOAEL: 0.50<br>LOAEL: 1.0 | NOAEL: 0.076<br>LOAEL: 0.10 |
| Day 42 Length | Mann-Whitney | NOAEL: 49<br>LOAEL: >49 | NOAEL: 1.0<br>LOAEL: >1.0 | NOAEL: 0.10<br>LOAEL: >0.10 |
| Day 42 Offspring/Fem | Jonckheere-Terpstra Step-Down | NOAEL: 11<br>LOAEL: 24 | NOAEL: 0.24<br>LOAEL: 0.50 | NOAEL: 0.044<br>LOAEL: 0.076 |
| Day 42 Cum. Off. | Jonckheere-Terpstra Step-Down | NOAEL: 11<br>LOAEL: 24 | NOAEL: 0.24<br>LOAEL: 0.50 | NOAEL: 0.044<br>LOAEL: 0.076 |
| Day 42 Male:female ratio | Mann-Whitney | NOAEL: 49<br>LOAEL: >49 | NOAEL: 1.0<br>LOAEL: >1.0 | NOAEL: 0.10<br>LOAEL: >0.10 |

**13. REVIEWER'S COMMENTS:**

The reviewer's conclusions were based on the time-weighted average concentrations in terms of the bulk sediment, OC-normalized sediment, and porewater. Therefore, the reviewer's results are reported in the Conclusions section of this DER. The reviewer's results generally agreed with the study author's. The only difference between the reviewer's and study author's conclusions was for the Day 28 length endpoint; the study author detected a significant reduction at the TWA 24 µg ai/kg level when the highest affected survival level (TWA 49 µg ai/kg) was omitted from the

length analysis. The reviewer did not omit any levels from the length analysis and did not detect any significant differences on this endpoint.

The reviewer's analysis detected significant reductions in cumulative number of offspring and offspring per female on Day 35 at the lowest treatment level (TWA 4.9 µg ai/kg) but not at the next higher level (TWA 11 µg ai/kg). Because there was not a clear dose-dependent pattern, and no differences were detected at the lowest level for these endpoints on Day 42, the reviewer concluded that the NOAEL was 11 µg ai/kg.

In addition to mean-measured sediment concentrations, results were reported (by the study author) in terms of OC-normalized sediment concentrations. The organic carbon content of the natural sediment was 4.8%.

| Nominal Sediment (µg ai/kg) | Mean-measured Sediment (µg ai/kg) | OC-Normalized Sediment (µg ai/g OC) |
|---|---|---|
| 6.3 | 5.0 | 0.10 |
| 13 | 11 | 0.23 |
| 25 | 22 | 0.46 |
| 50 | 47 | 0.98 |
| 100 | 91 | 1.9 |

The reviewer calculated the time-weighted average concentrations using the following equation:

$$C_{TWA} = \frac{\left(\frac{C_1 + C_0}{2}\right)(t_1 - t_0) + \left(\frac{C_2 + C_1}{2}\right)(t_2 - t_1) + \left(\frac{C_{n-1} + C_2}{2}\right)(t_{n-1} - t_2) + \left(\frac{C_n + C_{n-1}}{2}\right)(t_n - t_{n-1})}{t_n}$$

where:
C TWA is the time-weighted average concentration,
C j is the concentration measured at time interval j (j = 0, 1, 2,...n)
t j is the number of hours (or days or weeks, units used just need to be consistent in the equation) of the test at time interval j
(e.g., t 0 = 0 hours (test initiation), t 1 =24 hours, t 2 =96 hours)

Though the measured sediment concentrations were relatively stable over the duration of the exposure period (CV of <20% except in the nominal 6.3 and 25 µg ai/kg groups which had CVs of 28 and 29%, respectively), the measured porewater concentrations were highly variable (CV of 9.37-146%). For consistency, the reviewer calculated the sediment TWA concentrations, which were then corrected for the sediment organic carbon content (5.0%). Due to the high variability in porewater concentrations, there was a shift in the order of treatment levels with respect to nominal concentrations (TWA porewater concentrations were 0.045, 0.044, 0.076, 0.10, and 0.18 µg ai/L).

Sediment and pore water (exposure and QC) samples were analyzed using liquid chromatography with mass spectrometry (LC/MS/MS) and gas chromatography with mass selective detection (GC/MSD), respectively, based on methodology validated at Smithers Viscient. The sediment method validation study was conducted prior to study initiation and established an average recovery of 87.6 ± 7.44% from artificial sediment and 97.9 ± 11.6% from marine sediment. The aqueous matrix method validation was also conducted prior to study initiation and established an average recovery of 91.2 ± 4.98% from filtered seawater. It was reported that conditions and procedures used throughout the analysis of exposure solutions and QC samples during this study were similar to those used in the method validations.

Overlying water was not analyzed due to the pyrethroids' strong affinity to sediment (i.e., high Koc values) and regular renewal of the overlying water.

The dissolved organic carbon (DOC), total organic carbon (TOC), ammonia (as N), temperature and pH were measured in isolated pore water at each level on Days 0, 14, and 28. Throughout the study, the DOC ranged from 6.3 to 15 mg/L, the TOC ranged from 66 to 250 mg/L, the temperature was maintained at 21 to 22°C, and the pH ranged from 5.6 to 6.8. Ammonia decreased from 5.1 to 6.1 mg/L on Day 0 to 0.34 to 0.80 mg/L by Day 28.

The experimental phase of the definitive test was performed from July 25 to September 16, 2013.

## 14. REFERENCES:

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